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(54) Title: CARTILAGE EXTRACTION PROCESSES AND PRODUCTS (57) Abstract A biologically active cartilage product is prepared by heating a mixture of crude animal or fish cartilage in water under pressure to form an aqueous cartilage extract, removing suspended matter from the extract and concentrating the extract under vacuum. The product may be dried to form a fine granular material.		

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CARTILAGE EXTRACTION PROCESSES AND PRODUCTS1 Background of the Invention

5 This invention pertains to cartilage extraction processes and products. More specifically, the invention relates to a process for preparing a biologically active cartilage product using raw animal or fish cartilage together with adhering tissue as the starting material.

10 The preparation of powdered cartilage products for various therapeutic applications is discussed in the prior art. Thus, U.S. Patents 3,400,199 and 3,772,432 teach the preparation of pharmaceutical materials from raw cartilage. In the preparation of these prior art products it was considered important to remove the adhering tissue (mainly proteinaceous and fatty tissues) from the raw cartilage material by pre-treatment with a suitable proteolytic enzyme solution. Thus, the raw cartilage material might be treated with a solution of pepsin and an acid (e.g., acetic or hydrochloric) at a temperature of about 50°C for about five hours. After the adhering tissues had been removed the clean cartilage was vacuum

20 dried, de-fatted with a suitable solvent (e.g., hexane), the solvent evaporated and the cartilage mechanically comminuted to a fine powder of between about 5 to 40 microns average particle size. The cartilage powder so obtained could be utilized in powder form or, extracted at a temperature between about 3 to 4°C with distilled

25 water or an aqueous salt solution which facilitated solubilization or peptizing of the cartilage material at low temperature. The solubilized cartilage obtained in this manner is not a true solution but rather consists of a colloidal dispersion containing between about 1 and 10% cartilage solids.

30 Because of the complexity and number of steps required for preparation, cartilage products prepared according to the prior art processes are relatively expensive materials. The resulting materials also suffer from several significant drawbacks. Solutions containing solubilized cartilage materials are generally opaque. Although it has been recognized that the opacity is

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1 attributable to a small quantity of suspended particles
 (of larger than colloidal size) they are difficult to
 remove from cold solutions (either by centrifuging or
5 filtration) without simultaneously incurring substantial
 losses of active material. Since it was found that the
 presence of oxygen during the extraction process tended to
 degrade the resulting extract, and lower its biological
 activity, it was believed necessary to maintain a low
10 temperature oxygen-free environment during cartilage
 processing steps in order to avoid degradation and loss of
 biological activity in the finished product. An important
 element of prior art processing operations involved
 grinding the cartilage material to a predetermined average
 particle size, an expensive and time-consuming operation.

15 It has now been surprisingly discovered that
 cartilage preparations having high biological activity can
 be obtained by extracting crude, mechanically trimmed
 cartilage (i.e., cartilage which still retains a portion
 of the adhering tissues - primarily proteinaceous and
20 fatty tissues) together with water, under heat and pres-
 sure, for a predetermined time period. In contrast with
 prior processes, it has been unexpectedly discovered that
 cartilage materials need not be processed at low tempera-
 tures or in a completely inert, oxygen free atmosphere,
25 but can be processed at high temperatures (from about 50°C
 up to about 150°C) and pressures (between about 10 and
 about 60 PSI) to yield extracts possessing high biological
 activity. As a further advantage the process of the
 present invention dispenses with the requirement of a
30 pre-extraction grinding operation thereby substantially
 reducing processing costs. However, pre-extraction
 grinding can be optionally employed under certain process-
 ing conditions to yield a desirable product.

 According to the present invention, cartilage
35 products having high biological activity are prepared by
 heating a mixture of crude cartilage and water under
 pressure and substantially non-oxidizing conditions for a
 predetermined period of time to form an aqueous cartilage



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1 extract, removing suspended solid matter from the extract
and concentrating the extract under vacuum. As employed
herein the term "substantially non-oxidizing conditions"
5 refers to controlled environments in which small quanti-
ties, on the order of a few percent or less (by volume) of
air or oxygen may be present. The concentrated extract,
which may be in gel or liquid form, can be dehydrated
using any of several techniques, to yield a friable
10 granular material which forms clear or slightly hazy
solutions with water. The solid and liquid cartilage
products of the present invention are useful as ingredi-
ents in various pharmaceutical and cosmetic compositions.

It is accordingly an object of the present
invention to provide a process for preparing cartilage
15 products from raw cartilage.

Another aspect of the present invention relates
to the preparation of new cartilage products that are use-
ful in various pharmaceutical and cosmetic applications.

A still further aspect of the present invention
20 involves a method for using cartilage products prepared
according to the present invention in pharmaceutical
formulations for the treatment of pruritus ani and hemmo-
rhoidal conditions.

These and other objects of the present invention
25 will become apparent upon consideration of the following
detailed description of the invention.

The present invention involves a process for the
preparation of cartilage products from raw animal or fish
cartilage. As used herein, the term "raw cartilage"
30 refers to cartilage from which the adhering tissues
(primarily proteinaceous and fat) has not been separated
or removed. Bovine cartilage (especially bovine tracheal
cartilage) is the preferred raw material for use in the
invention, however cartilage taken from other vertebrate
35 animals including porcine and canine cartilage as well as
cartilage from the partly calcified skeleton, including
fetal skeleton, of very young or newly born animals will
also provide suitable results. Cartilage from young



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1 animals or young or newly regenerated cartilage from older
animals has also been found satisfactory for use in the
present invention. Cartilage from mature animals in
either the form which would in maturity retain the carti-
5 laginous form or which would in maturity ossify to bone
may also be employed. Skeletal cartilage from fish,
particularly the shark, has also been found to provide
an especially satisfactory raw material.

Cartilage from the skeletons of shark or other
10 cartilaginous fish may be used to prepare aqueous carti-
lage extracts in the same manner as bovine cartilage.
However, in the case of the shark, the spinal column is
the most convenient tissue to harvest. Despite the fact
that in most sharks the vertebrae are calcified to a
15 considerable degree, they contain sufficient cartilage
material to yield a useful extract. While bovine trachea
is the preferred source of raw cartilage, as it is the
most readily accessible cartilaginous tissue in mammals,
hyaline or costal cartilage from other parts of the
20 animal's body may be utilized to produce satisfactory
extracts.

The raw cartilage may be prepared by any satis-
factory means but generally is obtained by removing
substantially all of the skin, integument and organs of
25 the animal or fish and separating the cartilage. The
separated cartilage, to which some proteinaceous tissue
and fat will generally still be adhered may be subdivided
into chunks or used in whole form as removed from the
animal. It is not necessary to remove all vestiges of
30 adhering tissue, as in prior art cartilage processing
techniques. The size of the raw cartilage to be employed
in the invention is not critical and is primarily depen-
dent upon the dimensions of the reaction vessel in which
the cartilage is to be processed.

35 The raw cartilage is mixed with water in the
ratio from 1:100 to 100:1 and preferably 1:2 to 2:1 by
weight (cartilage:water). Preferably the water is deio-



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1 nized or distilled. The mixture of raw cartilage and
water is transferred to a suitable pressure vessel (e.g.,
a steam pressure vessel), fitted with a pressure relief
valve and the vessel heated to a temperature of between
5 50°C and 150°C and preferably between about 105°C and
125°C or until a pressure of 10 to about 60 p.s.i.g., and
preferably between about 10 to about 30 p.s.i.g. (pounds
per square inch - gauge reading) of steam has been built
up within the vessel. The preferred extraction condition
10 is 20 p.s.i.g. pressure for two hours (at a temperature of
about 110 degrees C). However, reaction periods of be-
tween 5 minutes and 5 hours may be employed to yield
satisfactory results. Under optimum conditions the ex-
traction is continued to the point at which the cartilage
15 substance has just become completely dissolved, but the
aqueous extract is still a light tan color and has not
acquired a dark brown color. The connective, fibrous and
fatty tissues adhering to the raw cartilage are extracted,
but not solubilized, during the heat and pressure treat-
20 ment.

Substantially all of the fat contained in the
cartilage, and adhering tissues, is released during the
extraction under heat and pressure, and floats to the
surface of the extract. The insoluble fibrous tissues
25 containing a minor portion of fat will generally sink to
the bottom of the reaction vessel.

At the conclusion of the heat and pressure
treatment, the vessel is opened and the aqueous cartilage
extract removed. The solid matter consisting primarily of
30 fibrous tissues and fat is separated from the extract by
centrifuging. In some instances, the solids may be
removed by decanting the extraction liquid. A small
portion of the fatty content of the raw cartilage remains
emulsified in the extract and may be removed either by
35 prolonged centrifuging at very high speeds (centrifugal
pressures of 1,000 G's or more). Alternatively, the
emulsified fat is removed by filtering through a filter



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1 press coated with a diatomaceous earth filter aid. The
filtrate (or the liquid recovered from the centrifuge) has
a clear amber color. Analysis of the recovered extract
shows it to be rich in proteinaceous material and low in
5 calories. The extract is suitable for use as a dietary
food supplement or a health food.

The filtered or centrifuged extract is concentrated by removing a portion of the moisture content under vacuum in a mechanically agitated thin film evaporator.
10 Thin film evaporator devices are well known in the art and utilizes rotating slotted wiper blades to generate a thin film of liquid on the heated wall of a sealed vessel. The slots in the rotating wiper blades provide a pumping action which creates and moves the thin liquid film along
15 a heated wall with constant agitation. As the concentrating residue travels downward, it is in continuous contact with the evaporating surface from which vapor is continuously separated. The vapor travels through a rotating entrainment separator to the surface of an
20 internal condenser where it is condensed and flows by gravity to an outlet valve. The action of the wiper blades in moving the residue down and off the heated wall eliminates thermal degradation by controlling the residence time at the distillation temperature. The
25 concentration is conducted under a vacuum of between about 10 and 100 tor (millimeters of mercury) and a jacket temperature of between about 90 and 180 degrees C.

A wiped film (or thin film) evaporator apparatus suitable for use in the present invention is available
30 from the Pfaudler Company, 1000 West Avenue, Rochester, New York as a 12 inch diameter model WFE with four square foot heating surface, Teflon wiper blades, Denison fluid motor MF05-014 and an 11 square foot internal condenser. The aqueous cartilage filtrate (or centrifugate) is
35 concentrated under vacuum, preferably of 40 tor (absolute) to yield a liquid of between about 45 to 65% solids content. The viscosity of the liquid will vary depending

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1 upon the temperature and solids content. Thus the liquid
may be readily flowable and of low viscosity at higher
temperatures and low solids content, and conversely may be
5 in gel form at low temperatures and with a higher solids
content.

The liquid concentrate (which is a partially de-
hydrated product) may be used as such in various cosmetic,
pharmaceutic and food products or it can be completely
dehydrated to a dry granular substance. Suitable dehydrat-
10 ing techniques include freeze drying, vacuum spray drying,
dehydration with a liquid (e.g., ethanol, isopropanol);
azeotropic distillation with a hydrocarbon azeotrope
(e.g., hexane). In making a product to be used in the
manufacture of an injectable cartilage extract it is
15 preferable to combine the liquid concentrate with an
excess of isopropanol to precipitate the active ingredi-
ents of the cartilage extract as a solid.

The completely dehydrated extract is a friable
granular or lumpy material which is generally of a light
20 tan color. The dry material can be readily ground to
yield a fine powder and dissolves readily to form clear or
slightly hazy solutions with water.

The cartilage extracts of the present invention
in either the dry or liquid form have been found to be
25 especially useful in the treatment of hemmorrhoidal condi-
tions and pruritus ani. The liquid extracts are also
useful as geriatric and dietary food supplements because
of their high protein and low fat content. The extracts
may be prepared in liquid or dry form for use as an in-
30 gredient (e.g., skin conditioner) in cosmetic formulations.

The biologically active agent of the invention
may be administered in the form of a liquid, as a suspen-
sion or solution, or alternatively in solid form as a
tablet pellet or capsule. The tablet may be prepared
35 using conventional tableting procedures in which the
active ingredient is combined with well known pharma-
ceutical excipients such as starch, sugar, bentonite clays



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1 and other commonly used carriers. Satisfactory pharma-
ceutically acceptable liquids include water, sugar solu-
tions, and aqueous glycols which may be compounded with
5 coloring agents and synthetic or natural flavors. In
another embodiment, the dry active ingredient may be in-
corporated onto silica gel or other gel forming materials
which are capable of coating the stomach walls. The
active ingredient may also be administered as a supposi-
10 tory, or a topically applied cream, or ointment. A
preferred embodiment of the invention useful for treating
hemorrhoidal conditions involves rectal administration of
the active ingredient in the form of a shaped suppository.

For the preparation of cartilage extracts that
may be administered by parenteral injection, it is desir-
15 able to remove as much of the adhering tissue and fat as
possible from the starting cartilage material prior to the
high pressure extraction step. This is accomplished by
mixing the raw cartilage in a digestive solution contain-
ing proteolytic enzymes (preferably acid-pepsin). Trypsin
20 and pepsin exemplify the wide variety of pro-eolytic
enzymes that are useful in this aspect of the invention.
Following the enzyme pretreatment, the digested cartilage
can then be extracted under high temperature and elevated
pressure. Although the pretreated materials or cartilage
25 extracts prepared can be used for food additives, cosmetic
applications and in topical and oral preparations, it is
not desirable to do so, as the enzyme pretreatment results
in the loss of some biologically active material from the
new cartilage. This loss occurs when the cartilage is
30 leached in the enzyme bath and during the subsequent
washing operations. Also, the pretreatment adds signifi-
cantly to the cost of the finished product.

In preparing a cosmetic composition in accord-
ance with the present invention, any suitable cream,
35 emulsion or oil cosmetic base, which will keep the cartil-
age material in solution or suspension may be used. The
base may be in emulsified form including waxes, oils,



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1 emollients, preservatives and humectants, or may be in the
form of a vegetable oil, or mixture of vegetable and
mineral oils or mixture of oil and water based composi-
tions.

5 The following examples illustrate certain
preferred embodiments of the invention. However, it
should be understood that these examples are non-limiting
illustrations and that other methods and embodiments are
envisioned by the present invention. Parts and ratios are
10 by weight except as otherwise stated.

EXAMPLE I

Extraction of bovine trachea.

5 kilograms of deionized water was admixed with
5 kilograms of well trimmed beef trachea subdivided into
15 pieces of about 3 inches in the largest dimension. The
water/trachea mixture was loaded into a 20 liter aluminum
pressure vessel equipped with a pressure gauge and a
pressure relief valve. The vessel was sealed, the lid
clamped shut, but the relief valve left open. The vessel
20 was heated to about 100°C to bring the water to a boil and
the relief valve left open until the steam generated by
the boiling water had displaced substantially all of the
air from within the vessel. The relief valve was then
closed and the steam pressure allowed to rise to about 20
25 p.s.i.g. (temperature 110°C) with the actual pressure
fluctuating between about 17 and 25 p.s.i.g. (105-116°C).
The pressure and temperature were held constant for two
hours. The vessel was then allowed to cool to atmospheric
pressure, the pressure valve opened and the lid of the
30 vessel removed. The partially processed trachea were
thoroughly mixed, the lid replaced, and the liquid again
brought to a boil. Air was purged from the vessel by
means of steam generation as outlined previously, the
pressure brought to 20 p.s.i.g. and held at that level for
35 another two hour period. The vessel was again cooled to
ambient temperature, the pressure valve opened, the lid
removed and the liquid contents of the vessel strained



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1 through a 100 mesh stainless steel strainer. The strained
liquid was centrifuged in order to remove the major part
of the suspended matter. The yield was as follows:

	Liquid Extract	7500 grams
5	Fat	850 grams
	Fibrous protein	
	matter with some fat	1600 grams

The fibrous matter residue contains 50% liquid
which was expressed to increase the yield of the liquid
10 extract. The liquid extract contains 8% non-volatile dry
extract which includes about 1% suspended fat (emulsified)
and protein. The remaining 7% represents 525 grams dry
weight, or 10.5% based on the weight of the trachea. The
liquid extract was a cloudy light tan colored fluid at
15 ambient temperature but consolidated to form a firm gel
upon refrigeration to 10°C or lower.

The liquid was found to be stable upon storage
under sterile conditions or when protected with a suitable
preservative (e.g., benzyl alcohol or a combination of
20 sorbic acid and sodium benzoate). The extract had a
pleasant taste, characteristic of meat extract and which
could be enhanced by seasoning with salt, pepper, or other
condiments customarily employed for seasoning soups or
meat products. The extract was suitable for use as a
25 dietary food supplement or could be administered as a
pharmaceutical preparation.

EXAMPLE II

The liquid extract of Example I was concentrated
to 45% non-volatile solids content under vacuum (40 mm of
30 Hg) and mechanical agitation at a jacket temperature of
120°C in a thin film evaporator (Pfaudler model WFE). The
liquid material formed a very firm gel when cooled to 15°C
or lower. The material had a pleasant taste and was in-
gested as a dietary food supplement. The gel was also use-
35 ful as a moisturizing component in conventional cosmetic
cream and ointment formulations.

EXAMPLE III

The concentrated extract (45% solids) of Example



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1 II was evaporated to dryness in a laboratory oven (110°C)
and under 40 millimeters of vacuum. The dried material,
which was hard and brittle, was pulverized. The pulver-
5 ized material was used to fill one thousand 50 milligram
hard shell gelatin capsules. The capsules were suitable
for oral administration. The powder was also used to make
shaped rectal suppositories for administration to humans
to alleviate pruritus ani and hemorrhoidal inflammation
(see Example IX).

10

EXAMPLE IV

2200 pounds of bovine trachea was quartered and
ground in a Weiler (meat) grinder using a one-half inch
orifice plate. After grinding, the material was trans-
ferred to a 100 gallon glass lined reactor equipped with a
15 mechanized agitator. The liquid-trachea mixture was
processed in six batches. The weight of trachea for each
batch varied between 300 and 425 pounds. Deionized water
was added to the reactor on a one-to-one weight ratio (1
pound of ground trachea to 1 part water). Internal
20 pressure of the reactor was brought to between 15 and 24
p.s.i.g. (temperature 110°C) after air purging and held
for two hours with the agitator in operation (120 r.p.m.).
The extracted mixture was removed from the pressure
chamber and pumped-through a centrifuge type decanter
25 (Flottweg Decanter Z-1L). The solids discharged from the
decanter were collected, weighed and sampled. The extrac-
tion liquid that remained after the decanting operation
was subjected to a three way separation in a Titan disc-
type centrifuge - the separations yielded:

30

- A. Light liquid - grease
- B. Heavy solids - sludge
- C. Water phase - product

The product from centrifuge batch 1 and 2 was further pro-
cessed through a filter press. The pH of the product
35 obtained from centrifuge batches 3 through 6 was adjusted
with glacial acetic acid to between about pH 5 and pH 5.5.
The Titan disc centrifuge had a bowl speed of 6200 r.p.m.
and an effective G force of more than 1,000 G's.



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1 The product from batches 1 and 2, after filtra-
 tion, was combined, the combined material decanted and
 centrifuged again in the disc centrifuge. Approximately
 1/2% of benzyl alcohol was added to the combined product
 5 as a preservative. The products of batches 3 and 4 were
 combined as were the products of batches 5 and 6. The
 following analyses were performed:

Raw Material (Material In) - in lbs.

10	Batch #	1	2	3	4	5	6
	Trachea	317	350	350	347	425	425
	Water	317	382	350	347	425	457
15	Total	634	732	700	694	850	882

Material Out - in lbs.

20	Batch #	1&2	3	4	5	6*
	Decanter Solids	81.5	40	41	53	84
	Cent. Eff. - Product	1034	523	543	613	686
	Grease	76	51	38	56	89
25	Cent. Sludge	19	None	21	None	20
	Total	1210.5	614	649	722	879

30 * Between Batch 5 and 6 the product from batch 1 and 2,
 which had been filtered and contained filter earth, was
 reprocessed thru the decanter and centrifuge. Therefore,
 the decanter solids for Batch 6 contained some filter
 earth.

35

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1 Material Balance

Batch	1 & 2		3 thru 6		1 thru 6	
	Lbs.	%	Lbs.	%	Lbs.	%
5 Material in (total)	1366	-	3126	-	4492	-
Material out (total)	1210.5	88.6	2864	91.6	4074.5	90.7
Assume lost as steam	155.5	11.4	262	8.4	417.5	9.3
Decanter solids	81.5	6.0	218	7.0	299.5	6.7
10 Cent. Eff. - Product	1034	75.7	2365	75.6	3399	75.7
Grease	76	5.6	234	7.5	310	6.9
Cent. sludge	19	1.4	41	1.3	60	1.3

Analysis: The following analyses were performed:

15 Moisture: Volatile Material - The samples were dried in a forced air oven set at 105°C.

Fat Analysis - The dried samples were fat extracted with petroleum ether in a soxhlet extraction tube for a minimum of 2 hrs.

20 Protein Analysis - Protein analysis on the dried, fat-free samples. The boric acid modification of the Kjeldahl method for the determination of nitrogen was used. The following factors were used:

$$25 \quad \% N_2 = \frac{\% NH_3}{1.2158}$$

Decanter Solids

Batch	1-2		3-4		5-6	
	Dry	As Rec.	Dry	As Rec.	Dry	As Rec.
30 Moist. & Vol.	0.0	73.8	0.0	72.3	0.0	72.3
% Solids	100.0	26.2	100.0	27.7	100.0	27.7
% Fat	7.6	2.0	8.9	2.5	6.7	1.9
35 % Protein	-	-	76.1	19.2	76.6	19.8



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1 Centrifuge Extract

Batch	1-2		3-4		5-6	
	Dry	As Rec.	Dry	As Rec.	Dry	As Rec.
5 Moist. & Vol.	0.0	83.7	0.0	83.3	0.0	84.6
% Solids	100.0	16.3	100.0	16.7	100.0	15.4
% Fat	5.7	0.9	6.6	1.1	3.6	0.6
% Protein	-	-	54.5	8.5	55.5	8.2

10

Approximately 100 gallons of liquid from centrifuge batches 1 and 2 were fed into the feed tank of a standard 1p faudler WFE 4 square foot wiped film evaporator set up with a louvered rotor having spring mounted wiper blades in which the outer jacket temperature was 173°C. The feed, a milky white, water-like liquid was heated by the outer jacket to 40.5° C and the internal operating pressure of the evaporator reactor adjusted to 40 millimeters of mercury (absolute). The liquid was

15 fed through the heated evaporator (173° C) at a feed rate of 225 pounds per hour to yield a 70% distillate split (i.e. 70% of the water in the starting material was removed).

20

EXAMPLE V

25	Blue shark spinal column	5305 grams
	Water, deionized	5400 grams
	Total charge	10,705 grams
	Yield	
	Liquid Extract	7300 grams
30	Insoluble calcified and proteinaceous residue	3000 grams

Process: Water was charged into a pressure vessel (as in Example I), heated to about 90° C and sections of a blue shark spinal column (frozen) then

35 added. The same heat and pressure extraction procedure was used as in Example I, but the total heat and pressure

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1 treatment time was only two hours as the shark cartilage
solubilized more quickly than the bovine cartilage.
The liquid extract was centrifuged and then conducted
5 through a filter press coated with a diatomaceous earth
filter aid to remove suspended solid materials. The
filtered extract had a light tan color and was almost
transparent being essentially fat free. The extract is
stable under sterile conditions or under ambient tempera-
10 ture conditions if suitable preservatives (e.g. benzyl
alcohol 1/2-1%) are incorporated.

EXAMPLE VI

The process of Example V was repeated using sand
shark spinal columns as the starting material. The yield
15 and quality of the extract was substantially identical to
that obtained in Example V.

EXAMPLE VII

Raw calf trachea was ground in a meat grinder to
20 a 5³ mm average particle size and loaded into an extrac-
tion vessel. The extraction vessel used was a 4 liter
jacketed stainless steel pressure vessel having a remov-
able top and equipped with a steam inlet, a steam outlet
valve, a pressure gauge, thermometer, a turbine mixer
25 operating at 120 r.p.m., and a material inlet capable of
introducing dry material against the prevailing pressure
in the vessel. The vessel containing 500 ml of de-ionized
water was preheated to the required temperature. The
specified pressure was obtained by introducing into the
30 vessel through the steam inlet valve. The air was sub-
stantially purged from the vessel with steam. The mixer
was activated and the 500 grams of ground cartilage forced
into the vessel with nitrogen. Satisfactory extracts were
obtained at the pressures (in absolute psi), temperatures
35 (in degrees Celsius), and extraction times (duration of



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1 treatment) given in the table below:

	<u>PSI</u>	<u>°C</u>	<u>Extraction Time</u>
	14.7	100	2 hours
5	20	109	1.5 hours
	25	116	1 hour
	30	121	30 minutes
	35	126	20 minutes
	40	131	15 minutes
10	50	138	10 minutes
	60	145	5 minutes

15

EXAMPLE VIII

The following tests were performed utilizing the
aforementioned vessel (Ex. VII) but replacing the turbine
mixer with a homogenizing type enclosed turbine mixer,
20 e.g. Barinco, Model CJ4A (Arde-Barencolo, Mahwak, New
Jersey), operated alternately in the upward mode and in
the downward mode at a transformer setting of 80 which
results in a speed of about 5000 r.p.m. In these tests,
the pressure in the vessel was not allowed to rise above
25 atmospheric pressure (14.7 p.s.i.) during extraction. The
extraction condition (temperature and time) were varied
for each extraction. The vessel was blanketed with an
inert gas or non-oxidizing gas (nitrogen). The calf
trachea was pre-ground to a 2³mm average particle size
30 to allow the mixer to operate at the closest rotor/stator
setting thereby providing the highest possible whearing
conditions. The following temperature/time schedules
yielded satisfactory (biologically active) extracts
under the above processing conditions.

35



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1	<u>°C</u>	<u>Time in Minutes</u>
	50	60
	75	30
	90	20
5	100	10

By using a suitable homogenizing mixer, e.g. Barinco, Tekmar, Eli Dicon, Eppenbach etc., and ground cartilage, it is possible to extract the cartilage in a continuous manner at elevated temperatures and at atmospheric or only slightly elevated pressures. The resulting extracts are a pale amber color and show only minimal heat degradation.

EXAMPLE IX

Twenty male and 20 female patients ranging in age between 60 and 85 years received a dietic food supplement consisting of cartilage extract obtained from Example I herein. All of the patients suffered from joint pains and limited joint movement attributable to confirmed rheumatoid arthritic conditions. Each patient received a daily dose of the liquid cartilage extract of Example I at the equivalent rate of 8 grams per day of dry extract for a period of three months. The daily dosage was subdivided into three approximately equal size portions.

Thereafter the patients each received the liquid extract at the equivalent of 6 grams per day of the dry extract (subdivided into two doses of 3 grams) for three months. Thereafter each patient received the equivalent of 4 grams per day of the dry extract for a subsequent three month period. The cartilage material was self-administered via the oral route.



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1 The physical condition of each patient was
checked prior to commencement of the trial, daily for the
first week, and once a week thereafter. Blood and urinal-
2 ysis tests were conducted on each patient prior to the
5 test to determine a baseline, and thereafter on a weekly
basis. All patients reported that movement in the affec-
ted joints became less painful and the range of motion
had increased between 7 and 30 days after commencement of
the cartilage regime. Patients who discontinued the
10 cartilage food supplement experienced a gradual return of
their previous arthritic pain level within three to eight
months. The periodic physical examinations and laboratory
tests confirmed that the cartilage diet had no adverse
effects on any patients' vital functions.

15

EXAMPLE X

 Rectal suppositories were prepared using a
20 hydrogenated vegetable oil base having a melting point of
about 37° C. Two groups of one thousand two inch supposi-
tories were prepared containing respectively 2%, and 5%
vacuum dried cartilage extract powders obtained in Example
III. The suppositories were administered to 40 patients
25 in a double-blind study. 20 patients with confirmed
hemorrhoidal lesions received active suppositories in two
groups. The first group received suppositories containing
2% by weight (40 mg) of dried cartilage extract and the
second group received suppositories containing 5% (100 mg)
30 active ingredient. The control group of 20 persons
received only a placebo consisting of a hydrogenated
vegetable oil base of the same shape, color, size and
weight as the active suppositories. Each patient received
three suppositories a day at approximately six hour
35 intervals for a period of three weeks. The test results
revealed that the cartilage extract containing (active)



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- 1 suppositories were highly effective in reducing the inflammation and pain of the hemorrhoidal lesion. Patients receiving the active suppositories experienced fewer bleeding episodes and experienced rapid healing of small
5 anal fissures. Those patients receiving placebo suppositories reported negligible effects attributable to the lubricating effect of the vegetable oil base.

EXAMPLE XI

- 10 Skin cream with cartilage extract as the active ingredient.

	<u>Composition:</u>	<u>% by weight</u>
	Lanolin-acetylated	2.50
	Petrolatum	4.00
15	Beeswax, bleached	2.50
	Cetyl alcohol	1.50
	Isopropyl myristate	11.00
	Stearic acid	1.50
	Mineral oil	9.20
20	Glycerol	3.00
	Borax	0.80
	Colloidal clay	2.00
	Triethanol-amine	1.40
	Sorbic acid	0.20
25	Benzyl alcohol	0.90
	Cartilage extract, dry. Example III	2.00
	Water, distilled	<u>57.50</u>
	Total	100.00

- 30 Procedure: The water was heated to about 80°C. The cartilage extract, borax, glycerol, triethanol-amine and sorbic acid were dissolved in the hot water. Using a high speed homogenizing mixer, the colloidal clay and stearic acid were dispersed. The balance of the ingredients were
35 then dispersed in the aqueous medium. The resulting cream was then allowed to cool to ambient temperature. Cosmetic

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1 creams exhibiting high moisture retention qualities in the
 skin were obtained when 2 to 4% by weight of cartilage
 extract solids were incorporated into the cream. Similar
 results were obtained when the cartilage extract employed
 5 was a liquid extract of 8% solids content (as in Example
 I) the 45% concentrate of Example II or the extracts,
 liquid or dried, obtained in Example III and IV. The
 shark cartilage of Example VII was used after it was
 deodorized by steaming.

10

EXAMPLE XII

Shaped lipsticks were prepared utilizing conven-
 tional techniques and standard cosmetic bases comprising
 hydrogenated vegetable oils, lanolin waxes, lanolin
 alcohols, beeswax and petrolatum. Into such bases carti-
 15 lage extracts were incorporated in quantities of 2 to 10%
 by weight based on the dry extract (prepared as in Exh.
 III). The lipsticks were pleasant on the lips and had an
 effective emollient action. In use they effectively
 prevented development of chapped lips and hastened the
 20 healing of cracked lips. The therapeutic lipsticks also
 accelerated the healing of herpes simplex lesions ("cold
 sores") on the lips.

EXAMPLE XIII

The following cosmetic facial creams were
 25 prepared utilizing the cartilage extract of Example I.

CREAM A

		<u>%</u>	<u>Gr.</u>
	(I) Modulan (Acetylated Lanolin - Amerchol Corp.)	3.0	600
30	White petrolatum	5.0	1000
	Beeswax	3.0	600
	Isopropyl Myristate	8.5	1700
	Cetyl alcohol	2.0	400
	White mineral oil	6.5	1300
35	Stearic Acid	2.0	400
	Oil base	30.0	6000



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1		<u>%</u>	<u>Gr.</u>
	(II) Distilled water	35.0	7000
	Borax	0.7	140
	Veegum (colloidal clay -	1.5	300
5	R.T. Vanderbilt Co.)		
	Titanium dioxide	.2	40
	Triethanolamine	.2	40
	Cartilage extract (Ex. I)	1.2	240
	Benzyl Alcohol	25.0	5000
10	Glycerol	.9	180
		<u>5.3</u>	<u>1060</u>
	Water base	70.0	14000

15 I and II were prepared separately. Both were heated to 70°C and I slowly added to II under constant mixing of II with a Barinco mixer (Arde-Barinco Corp., Mahwah, N.J.).

20 The resulting material, a cosmetic cream of uniform consistency, was packed in 50 ml glass bottles and the bottles sealed with threaded covers.

CREAM B

25 -	(I) Modulan	300
	White Petrolatum	500
	Beeswax	300
	Isopropyl myristate	870
30	White mineral oil	700
	Stearic Acid	<u>200</u>
	Oil base	2870

35



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1	(II) Deionized Water	3500
	Titanium Dioxide	80
	Sorbic Acid	20
	Carbopol 934	46
5	Urea	300
	Triethanolamine	125
	Benzyl alcohol	80
	Cartilage extract (Ex. I)	<u>2500</u>
	Water Base	6651

10

II was placed in a vessel equipped with a Barinco Mixer, deionized water added and the mixture heated to 70-80°C followed by addition of TiO₂, sorbic acid and Carbopol. The ingredients were mixed to obtain a uniform dispersion. Urea was then added and mixing continued while the Triethanolamine, cartilage extract and benzyl alcohol were added. Then under constant mixing molten (I) was added and the combined ingredients mixed until a smooth homogeneous emulsion was obtained.

20

Cream B was packaged in 50 ml bottles, and the bottles sealed with threaded covers.

Both creams (A&B) were topically applied on a daily basis to the facial area by a group of 20 women for a period of about 3 months. There was a general increase in turgor of the skin and all 40 participants (20A and 20B) reported satisfactory skin toning and moisturization effects with the respective cosmetic formulations.

EXAMPLE XIV

30

Extraction of bovine trachea -
Ratio of water to trachea 1/100

50 grams of dionized water was admixed with 5 kilograms of well trimmed beef trachea that had been subdivided into pieces of about 1 inch in the largest dimension. The water/trachea mixture was loaded into a

35

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1 jacketed stainless steel pressure vessel having a 20 liter
capacity. The vessel was equipped with a variable speed
stainless steel turbine mixer, a steam inlet valve,
theremometer, pressure gauge and pressure relief valve.
5 The vessel was sealed, the lid clamped shut and the relief
valve left open. Steam was introduced into the jacket and
the mixer activated at a speed of about 20 RPM. When the
temperature in the vessel reached 100°C the steam valve
leading into the vessel was opened and a slow stream of
10 moist steam introduced and kept on for about three minutes
until substantially all of the air in the vessel had been
purged. Both the steam valve and the pressure relief
valve were then closed and the steam pressure in the
jacket increased to 23 p.s.i.g. This brought the internal
15 pressure in the vessel to 20 p.s.i.g. This pressure was
maintained for 4 hours and the speed of the mixer in-
creased to 60 RPM after the first 30 minutes. The vessel
was cooled to ambient temperature by circulating cold
water through the jacket. The pressure valve was opened,
20 the lid removed and the contents of the vessel strained
through a 100 mesh stainless steel strainer while they
were still hot (90°C). The fibrous matter retained on the
strainer was stripped of the major part of the absorbed
liquor by compressing it in a Carver press at 5 p.s.i.
25 pressure. The liquor obtained at this step was combined
with the strained liquor and the combined liquors centri-
fuged to strip them of suspended matter. The solid
residue from the centrifuge was combined with the residue
from the Carver press. The yield was as follows:

30	Liquid Extract	2,500 grams
	Fat	840 grams
	Fibrous protein matter	
	some fat and moisture	1,550 grams
	Operation loss	160 grams

35



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1 The liquid extract contains 25% non-volatile dry matter
which includes about 2% fat (emulsified and insoluble
protein). The remaining 23% represents 575 grams dry
weight, or 11.5% based on the weight of the dry trachea.
5 The liquid extract was cloudy, had a light tan color which
gelled upon refrigeration to about 12°C. Except for its
higher solids content and somewhat higher viscosity,
the liquid extract was very similar to the extract of
Example I. When freeze dried, the product was essentially
10 identical to that obtained in Example I. In this Example
the bound water, locked in the cartilage tissue was
utilized in the extraction. The high solids content of
the liquor obtained makes this process the most economical
as less water has to be removed in the drying step.

15

EXAMPLE XV

Extraction of bovine trachea.

Ratio of water to trachea 100/1

5 kilos of deionized water was admixed with 50
grams of well trimmed beef trachea subdivided into pieces
20 of 1 inch in the largest dimension. The pressure vessel
and procedure were the same as in Example I. The yield of
liquid extract was 4,950 grams. The extract was hazy,
slightly opalescent, had a mild taste and did not gel,
even after refrigeration at 3°C. When freeze dried,
25 the product was very similar in physical characteristics
to that obtained in Examples I and I-A.

EXAMPLE XVI

Extraction of bovine trachea suitable
for injectable preparations

30 The following ingredients were used in an Enzyme
pretreatment operation to remove adhering tissues prior to
extraction of the cartilage tissues.

	Bovine trachea, well trimmed	3,000 grams
	Water, deionized	6,790
35	Acetic acid	180
	Pepsin	30



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1 The trachea and the water were loaded into a jacketed
stainless steel vessel equipped with a variable speed
stainless steel turbine mixer. The acetic acid and pepsin
were added with the mixer operating at 60 RPM. Hot water
5 was introduced into the jacket, the temperature of the mix
in the vessel raised to 55°C and held at that temperature
until the cartilage rings of the trachea were free from
adhering tissues (about 5 hours). The liquid, containing
the hydrolyzed tissues and most of the fat, was then
10 drained off. The residual trachea and cartilage rings,
were washed twice with deionized water (80°C) and twice
with cold deionized water until the pH of the last wash
above pH5.0. The yield was

15	Trachea-cartilage rings	1,500 grams
	Deionized water	1,500 grams

The trachea cartilage rings and deionized water were
loaded into a stainless steel pressure vessel equipped
with a variable speed stainless steel turbine mixer, a
steam inlet valve, thermometer, pressure gauge and
20 pressure relief valve. The vessel was sealed, the lid
clamped shut and the relief valve left open. Steam
was introduced into the jacket and the mixer activated at
60 RPM. When the water in the vessel began to boil, the
steam generated was allowed to purge the air from the
25 vessel; the relief valve was then closed. While the
mixing continued, the internal pressure in the vessel was
allowed to rise to 20 p.s.i.g. and maintained at about
this pressure for 2-1/2 hours. The steam in the jacket
was then replaced with cooling water and the temperature
30 in the vessel brought down to ambient temperature (about
17°C). The material in the vessel was blanketed with
nitrogen and 30 grams of a diatomaceous earth (acid washed
Hyflo Supercel) filter aid was introduced into the vessel
and the mixing continued for another 15 minutes. The
35 batch was then filtered through a ceramic filter precoated
with the same diatomaceous filter aid.



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1 The filtrate was concentrated to 25% of its
volume under vacuum with maximum pot temperature of 75°C.
The concentrated filtrate was introduced, under vigorous
agitation, into ten times its volume of isopropanol. The
5 precipitated and partially dehydrated cartilage substance
was filtered, washed with isopropanol and then with
acetone and finally stripped of the solvents in a forced
air dryer at 50-45°C until the loss on drying was less
than 2% of the dry weight of the substance.

10 The procedure yielded a product that was 5.5% by
weight of the crude trachea or 12.4% by weight of the
cartilage rings after the enzyme treatment. The material
was light gray-tan in color and was readily soluble in
water of ambient temperature, forming a light tan colored
15 solution which had a slight haze. (When the solution was
filtered through a Milipore filter a sparkling clear
solution suitable for use in formulating injectable
preparations for clinical administration to animals
or humans.

20

EXAMPLE XVII

The extraction was carried out as in Example
III, but the concentrated filtrate in this case was
dehydrated azeotropically using heptane as the azeotrope.
The dehydrated material was stripped of the solvent in a
25 forced air dryer at 45°C until the weight loss on drying
was less than 2%. The dried material was a granular
friable solid which had no perceptible solvent odor
and which was soluble in water at ambient temperature
forming a slightly hazy light amber colored solution.
30 The solution could be clarified via filtration.

EXAMPLE XVIII

The extraction process as in Example XV, but
the concentrated filtrate in this case was dehydrated by
35 freeze drying in a VirTis 25-SRC sublimator at a shelf



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- 1 temperature of +30°C and a vacuum of 45 microns. The
dried material was a flaky and very fluffy product of
light amber color, soluble in water at ambient temperature
forming a slightly hazy solution which could be readily
5 clarified by filtration or by centrifuging.



What is claimed is:

1. The method of producing a biologically active cartilage product which comprises:

admixing raw cartilage derived from a member selected from the group consisting of a cartilage bearing animal, fish or reptile with water in the ratio from about 1:100 to 100:1, cartilage to water,

heating said admixture to a predetermined pressure at a temperature in the range between about 50°C and 125°C,

maintaining said admixture at said predetermined pressure and temperature for a period of time between about 5 minutes and five hours to form an aqueous extract containing suspended solid materials, and

separating said suspended solid materials from said extract.

2. The method of claim 1 which comprises concentrating said extract under a vacuum after removing said suspended solid matter, said concentrated extract having a solids content of between about 45% and 65%.

3. The method of claim 2 wherein said pressure is between about 10 and about 30 pounds per square inch.

4. The method of claim 1 wherein said time period is between about 1 and 5 hours.

5. The method of claim 4 which comprises drying the concentrated extract.

6. The method of claim 5 wherein said drying step comprises said admixing said concentrated extract with a dehydrating liquid.

7. The method of claim 6 wherein said drying step comprises removing moisture from said concentrated cartilage extract by subjecting said extract to a temperature below 0°C in the presence of a vacuum.



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8. The method of claim 5 which comprises concentrating said liquid cartilage extract under a vacuum of between about 10 and 100 tor.

9. The method of claim 5 which comprises drying said cartilage extract by azeotropic distillation with a hydrocarbon azeotropic substance.

10. The method of claim 2 wherein said raw cartilage includes a small quantity of fat and adhering proteinaceous tissue.

11. The method of claim 5 wherein said raw cartilage is derived from a cartilage bearing animal.

12. The method of claim 11 wherein said animal is of the bovine genus.

13. The method of claim 6 wherein said raw cartilage is derived from a fish.

14. The method of claim 13 wherein said fish is a shark.

15. The method of claim 13 wherein said concentrated cartilage extract is a gel at room temperature.

16. A biologically active cartilage product which comprises a dry cartilage material prepared by separating substantially all of the organs, skin and integument from the cartilage of an animal, reptile or fish, admixing said raw cartilage with water in the ratio 1:100 to 100:1 cartilage to water, extracting said mixture of crude cartilage and water under elevated temperature and pressure for a predetermined time period to form an aqueous cartilage extract containing suspended solid material, separating the solid material from said aqueous extract, concentrating the aqueous extract under a vacuum and drying said concentrated extract to a dry product.



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17. The product of claim 16 wherein the concentrating step is conducted by agitating said extract in a thin film evaporator device.

18. The method of claim 1 which comprises digesting said cartilage in an acid-pepsin solution prior to said extraction under heat and pressure.

19. The method of producing a biologically active cartilage product which comprises:

admixing raw cartilage which has not been subjected to acid pepsin digestion with water, said admixture being in the ratio from 1:100 to 100:1 cartilage to water,

heating said admixture to a temperature of between about 50°C and 125°C under a pressure of between about 10 and about 30 pounds per square inch,

maintaining said admixture at said temperature and pressure for between about 5 minutes and 5 hours to form an aqueous extract containing suspended solid materials, and separating said suspended solid materials from said extract.

20. A shaped lipstick comprising the dried product of claim 19 and at least one lipstick base selected from the group consisting of hydrogenated vegetable oils, lanolin waxes, lanolin alcohols, beeswax and petroleum.

21. A shaped rectal suppositor comprising the biologically active cartilage product of claim 19, and a hydrogenated vegetable oil base.

22. The method of claim 21 which comprises separating said suspended solid materials from said extract by filtering said extract through a filter press.

23. The method of claim 21 which comprises separating said suspended solid materials from said extract by centrifuging said extract.



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24. The method of claim 21 which comprises concentrating said extract to dryness by dehydration, and grinding said dry material to a fine powder.

25. A pharmaceutical formulation comprising a pharmaceutically acceptable liquid and the biologically active cartilage product of claim 21.

26. The method of claim 2 which comprises combining said concentrate with an excess of isopropanol to precipitate a solid material.



INTERNATIONAL SEARCH REPORT

International Application No. **PCT/US80/00503**

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ³ According to International Patent Classification (IPC) or to both National Classification and IPC Int. Cl. A61K 7/025; A61K 35/12, 35/56 U.S. Cl. 424/64; 424/95		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
U.S.	424/64, 95	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁵		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category ⁶	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X	US, E, Re. 28093, 30 July, 1974, Column 5, lines 64-75	1-26
X	US, A, 3,400,199, 3 September, 1968, Column 2, lines 42-56	1-26
X	US, A, 3,476,855, 4 November, 1969, Column 2, lines 52-70	1-26
X	US, A, 3,478,146, 11 November, 1969, Column 2, lines 50-70	1-26
X	US, A, 3,772,432, 13 November, 1973, Column 5, lines 33-51	1-26
X	US, A, 3,966,908, 29 June 1976, Column 4, 22-55	1-26
X	CH, A, 476,495, 30 September, 1969, Column 5, lines 34-50	1-26
X	FR, A, 3,186M, 15 March, 1965, Column 2, lines 1-34	1-26
⁹ Special categories of cited documents: ¹⁹ <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ "A" document defining the general state of the art</p> <p>¹¹ "E" earlier document but published on or after the international filing date</p> <p>¹² "L" document cited for special reason other than those referred to in the other categories</p> <p>¹³ "O" document referring to an oral disclosure, use, exhibition or other means</p> </div> <div style="width: 45%;"> <p>¹⁴ "P" document published prior to the international filing date but on or after the priority date claimed</p> <p>¹⁵ "T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention</p> <p>¹⁶ "X" document of particular relevance</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ²		Date of Mailing of this International Search Report ²
18 August 1980		03 SEP 1980
International Searching Authority ¹		Signature of Authorized Officer ²⁰
ISA/US		<i>Gale L. Ore</i> DROre

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X

N, Archives of Surgery, issued 1 January, 1963, John F. Prudden et al, The Acceleration of Wound Healing, Vol. 86, See pages 157-161

1-26

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹⁰

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter ¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹³, specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ¹¹

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

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